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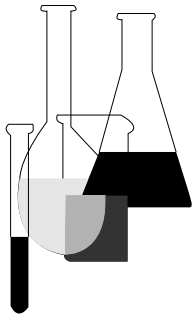
Health Effects Test Guidelines

OPPTS 870.5200 Mouse Visible Specific Locus Test



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading ''Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines.''

OPPTS 870.5200 Mouse visible specific locus test.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are OPPT 40 CFR 798.5200 Mouse visible specific locus test and OPP 84-2 Mutagenicity Testing (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982.

(b) **Purpose.** The mouse visible specific locus test (MSLT) may be used to detect and quantitate mutations in the germ line of a mammalian species.

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definitions also apply to this test guideline.

Germ line is the cells in the gonads of higher eukaryotes which are the carriers of the genetic information for the species.

Visible specific locus mutation is a genetic change that alters factors responsible for coat color and other visible characteristics of certain mouse strains.

(d) **Test method**—(1) **Principle.** (i) The principle of the MSLT is to cross individuals who differ with respect to the genes present at certain specific loci, so that a genetic alteration involving the standard gene at any one of these loci will produce an offspring detectably different from the standard heterozygote. The genetic change may be detectable by various means, depending on the loci chosen to be marked.

(ii) Three variations of the method currently exist for detecting newly arising point mutations in mouse germ cells:

(A) The visible specific locus test using either five or seven loci.

(B) The biochemical specific locus test using up to 20 enzymes.

(C) The test for mutations at histocompatibility loci.

(iii) Of the three tests, the visible specific locus test has been most widely used in assessing genetic hazard due to environmental agents. It is the method described in this guideline.

(2) **Description.** For technical reasons, males rather than females are generally treated with the test agent. Treated males are then mated to females which are genetically homozygous for certain specific visible marker

loci. Offspring are examined in the next generation for evidence that a new mutation has arisen.

(3) **Animal selection**—(i) **Species and strain.** Mice should be used as the test species. Male mice should be either $(C_3H \times 101)F_1$ or $(101 \times C_3H)F_1$ hybrids. Females should be T stock virgins.

(ii) **Age.** Healthy sexually mature animals should be used.

(iii) **Number.** A decision on the minimum number of treated animals should take into account the spontaneous variation of the biological characterization being evaluated. Other considerations should include:

(A) The use of either historical or concurrent controls.

(B) The power of the test.

(C) The minimal rate of induction required.

(D) The use of positive controls.

(E) The level of significance desired.

(iv) **Assignment to groups.** Animals should be randomized and assigned to treatment and control groups.

(4) **Control groups—**(i) **Concurrent controls.** The use of positive or spontaneous controls is left to the discretion of the investigator. However, any laboratory which has had no prior experience with the test should, at its first attempt, produce a negative control sample of 20,000 and a positive control, using 100 mg/kg 1-ethylnitrosourea, in a sample of 5,000 offspring.

(ii) **Historical controls.** Long-term, accumulated spontaneous control data of 43/801,406 are available for comparative purposes.

(5) **Test chemicals—**(i) **Vehicle.** When possible, test chemicals should be dissolved or suspended in distilled water or isotonic saline buffered appropriately, if needed, for stability. Water-insoluble chemicals should be dissolved or suspended in appropriate vehicles. The vehicle used should neither interfere with the test compound nor produce major toxic effects. Fresh preparations of the test chemical should be employed.

(ii) **Dose levels.** Usually, only one-dose level need be tested. This should be the highest dose tolerated without toxic effects, provided that any temporary sterility induced due to elimination of spermatagonia is of only moderate duration, as determined by a return of males to fertility within 80 days after treatment. For evaluation of dose-response, it is recommended that at least two dose levels be tested.

(iii) **Route of administration.** Acceptable routes of administration include gavage, inhalation, admixture with food or water, and IP or IV injections.

(e) **Test performance**—(1) **Treatment and mating**. Hybrid F_1 ($C_3H \times 101$) or ($101 \times C_3H$) male mice should be treated with the test substance and immediately mated to virgin T stock females. Each treated male should be mated to a fresh group of two to four virgin females each week for 7 weeks, after which he should be returned to the first group of females and rotated through the seven sets of females repeatedly. This mating schedule generally permits sampling of all postspermatagonial stages of germ cell development during the first 7 weeks and rapid accumulation of data for exposed spermatagonial stem cells thereafter. Repeated mating cycles should be conducted until the entire spermatogonial cycle has been evaluated and enough offspring have been obtained to meet the power criterion of the assay.

(2) **Examination of offspring.** (i) Offspring may be examined at (or soon after) birth but must be examined at about 3 weeks of age at which time the numbers of mutant and nonmutant offspring in each litter should be recorded.

(ii) Nonmutant progeny should be discarded. Mutant progeny should be subjected to genetic tests for verification.

(f) **Data and report—**(1) **Treatment of results.** Data should be presented in tabular form and should permit independent analysis of cell-stage specific effects and dose-dependent phenomena. The data should be recorded and analyzed in such a way that clusters of identical mutations are clearly identified. The individual mutants detected should be thoroughly described. In addition, concurrent positive and negative control data, if they are available, should be tabulated so that it is possible to differentiate between concurrent (when available) and long-term accumulated mutation frequencies.

(2) **Statistical evaluation.** Data should be evaluated by appropriate statistical methods.

(3) **Interpretation of results.** (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of specific locus mutations. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of specific locus mutations or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) **Test evaluation.** (i) Positive results in the MSLT indicate that under the test conditions the test substance induces heritable gene mutations in the test species.

(ii) Negative results indicate that under the test conditions the test substance does not induce heritable gene mutations in the test species.

(5) **Test report.** In addition to the reporting requirements as specified under 40 CFR part 792, subpart J, the following specific information should be reported:

(i) Strain, age, and weight of animals used, number of animals of each sex in experimental and control groups.

(ii) Test chemical vehicle, doses used, rationale for dose selection, and toxicity data.

(iii) Route and duration of exposure.

(iv) Mating schedule.

(v) Time of examination for mutant progeny.

(vi) Criteria for scoring mutants.

(vii) Use of concurrent or negative controls.

(viii) Dose response relationship, if applicable.

(g) **Additional requirements.** Testing facilities conducting the mouse visible specific locus test in accordance with this section should, in addition to adhering to the provisions of 40 CFR 792.190 and 792.195 obtain, and retain for at least 10 years, acceptable 35–mm color photographs (and their negatives) demonstrating the visible mutations observed in mutant animals and the lack of such mutations in their siblings and parents.

(h) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Russell, L.B. et al. The mouse specific locus test with agents other than radiations: interpretation of data and recommendations for future work: A report of the U.S. EPA's Gene-Tox Program. *Mutation Research* 86:329–354 (1981).

(2) [Reserved]